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Graduate Studies in Chemistry and Biochemistry

In Concordia University's Department of Chemistry and Biochemistry graduate students have the opportunity to develop close working relationships and a strong sense of community with faculty members. Because of the Department's reputation for quality research, it receives over a million dollars a year in external funding. Full-time graduate students who work as teaching or lab assistants receive approximately eleven thousand dollars a year. Faculty members, along with their research teams, are investigating a very broad range of areas. The Department is particularly strong in biochemistry, analytical chemistry, catalysis, materials, laser spectroscopy and photochemistry.

Chemistry researchers *(from left to right):* Paul Joyce, Peter Banks, Ann English, Youla Tsantrizos, John Capobianco and Nick Serpone.

Environmental Photochemistry

Professor **Nick Serpone** does both fundamental and applied research, primarily in the area of environmental photochemistry (and in imaging science). He uses a technique called heterogeneous photocatalysis to convert organic pollutants into carbon dioxide and to selectively recover dissolved metals into their metallic, non-toxic form.

"In the applied area, after ten years of experience we know that this novel technology known as heterogeneous photocatalysis works. We've tried a whole variety of compounds: herbicides, pesticides, dioxins, PCBs — you name it. As long as it is organic, we can destroy it," Serpone says.

The process works through the interaction of photons (light) with a semiconductor called titania (or titanium dioxide). Titania is a completely non-toxic substance found in household items such as toothpaste and white paint. When it is placed in water, the interaction between this semiconductor and the light photons generates electrons and holes (the latter are simply sites deficient of electrons) on the surface of the titania particles. The holes then oxidize the water molecules surrounding the particles, forming hydroxyl radicals. In turn these radicals attack the organic pollutants, turning them into carbon dioxide.

Serpone says he is convinced that there are no adverse environmental effects caused by the process: "Driving your car causes more damage than I do in destroying the pollutants." Metals cannot be destroyed, but through this technology they can be reduced catalytically from dissolved metal wastes into recoverable chunks of metal. "In the metallic state these are, of course, non-toxic except for mercury. As finely divided particles they may be toxic," Serpone states.

In order for the technique to work it is important that the electrons (e^-) and the holes (h^+) be kept apart. Using Concordia's Centre for Picosecond Laser Spectroscopy (founded in 1981 by Serpone), Serpone has determined that the two charged species (e^- and h^+) can recombine in as little time as one picosec-

ond, that is in a millionth of a millionth of a second. "Chemistry, is slow; recombination is fast," Serpone explains. "The chemistry has to compete with that recombination. To make it compete you can do two things: you can slow down the recombination or increase the efficiency of the chemistry."

For several years Serpone, who has been at Concordia since 1968, has been trying to do just that. He has discovered that placing metal ions inside the lattice of the titania particles causes them to "take the electron out of the picture momentarily. So that what I have left is a larger number of holes, which are nothing more than hydroxyl radicals, as long as I can get rid of the electrons. The more I have of these holes, the more destruction I will get, and the faster that destruction will be." In some cases, this metal-doping technique slows down the recombination process to within milliseconds rather than picoseconds.

Serpone is also examining new ways of making the titania — with sol-gels, for example — and exploring the effects of using two coupled semiconductors.

He first began working on heterogeneous photocatalysis in 1983 as part of a research team during a sabbatical leave in Switzerland. Initially, his interest was on splitting water molecules. They were hoping to access a cheap source of hydrogen that could then be used as fuel. While they succeeded in splitting the water molecules, the process only produced hydrogen at the rate of a few microliters per hour. But Serpone, among others, realized that the technique had potential applications in the destruction of organic pollutants.

The emerging technology is currently being made available on the marketplace. A chemist who worked with Serpone as a post-doctoral fellow is marketing a form of titania that could be used by companies to reduce industrial pollutants at source. Serpone himself, along with another Professor in the United States formed a small company in the U.S. in attempts to market the technology. If costs come down, the process could also be safely and effectively be used on municipal water supplies.

'The cooperation between the Department of Chemistry and the Centre for the Study of Behavioural Neurobiology is indicative of the growing interdisciplinary nature of studies in the neurosciences — studies that cross the traditional boundaries of chemistry, psychology, and biology.'

Laser Spectroscopy

Associate Professor **John Capobianco** and his group conduct research in the field of optical spectroscopy of condensed matter using different laser spectroscopy techniques. Optical spectroscopy of condensed matter presents a daunting challenge, requiring an understanding of the structure of solids, perfect and imperfect, of the electrons in solids and of the nature of elementary excitation in solids. The attraction of the study of this spectroscopy is the insight it affords into fundamental solid state processes. It is also an area of major technological interest which ensures a good measure of research support and continues to be a dynamic field of scientific enquiry.

Capobianco, who describes his research area as chemical physics, says his work could lead to the development of solid-state tunable lasers in the near infrared region. The interest in laser operating in the near infrared region ($\lambda > 1.4$um) stems from the fact that the laser would be eye safe and tunability in the 1.4 - 1.5um band would make it an ideal source for remote sensing of the atmosphere. Other possible applications would be in optical communications and laser surgery. He stresses however that "before you do any of this you must know the basic spectroscopic properties of the material."

His work is funded by the Natural Science & Engineering Research Council and the Department of National Defence's Research Centre in Val Cartier, Québec. Capobianco says that usually conjures up an image of "making a big powerful laser to shoot down missiles." In fact, the grant from National Defence is geared towards helping his team do research into eye safe lasers.

Since 1986, when he came to Concordia, Capobianco has worked hard to build up his spectroscopy lab. "We have facilities that can compete with other labs across Canada. Presently, we can do spectroscopy both in the visible and the near infrared region of the spectrum. So we are covering a very large region — that is something which not too many labs have," he states.

The facility includes various types of lasers, such as, a solid state tunable titanium sapphire, an argon ion, pulsed nitrogen and dye lasers. In addition, the lab has a cryogenic facility that attains temperatures as low as 4 Kelvin, or -273^{o}C.

Capobianco sees laser spectroscopy as an attractive area for many top-notch students because "they can do both experimental and theoretical work. Some students like to combine the two — they seem to flourish on being able to do both aspects of the science".

Neurochemistry

Adjunct Professor **Dorothy Pocock**, has a background in biology and biochemistry and is now working on the neurochemistry of the brain. She is Research Associate to Psychology Professor **Roy Wise** of Concordia's Centre for the Study of Behavioral Neurobiology (CSBN). Although Pocock has worked with Wise since 1986, formal collaboration between the Department of Chemistry and Biochemistry and the CSBN began in 1991. This cooperation is indicative of the growing interdis- ciplinary nature of studies in the neurosciences — studies that cross the traditional boundaries of chemistry, psychology, and biology.

"What's happening here is a sign of the times. The amount of information about how the brain works is just exploding. There are new discoveries every month," Pocock said. "In our own small way, with this collaboration between departments, we're doing what universities with medical faculties do on a large scale."

Pocock uses microdialysis in conjunction with high-performance liquid chromatography (HPLC) to monitor changes in the concentrations of particular neurotransmitters in the brains of rats. A cannula with a tiny dialysis membrane glued to its tip is inserted into a specific place in the brain. Fluid can then be perfused into the cannula, past the dialysis membrane and out a smaller exit tube. As the fluid passes by the dialysis membrane, molecules enter into it from the brain. Thus changes in the concentrations of molecules in the extracellular fluid in the brain can be monitored. The reverse process is also possible: drugs added to the perfusing fluid will go through the dialysis membrane into the brain. The fluid that comes out the exit tube can be injected directly into a HPLC system, without extraction or filtration.

"The beauty of this technique is that we can quantify changes in the neurochemistry of the brain in conscious, behaving rats," states Pocock. "You can put the rats in certain behaviour paradigms, for example brain-stimulation-reward, and measure changes in the levels of the substance of interest in the brain at the same time," she says. Thus changes in neurochemistry can be correlated with changes in behaviour.

The collaboration with the CSBN offers a unique opportunity for graduate students in the Chemistry and Biochemistry Department. Professor Wise is known internationally for his research towards the understanding of neural mechanisms underlying motivation. "Chemistry students coming into this group do not get involved in the behaviour aspect — because they don't have the psychology background for it. However, there's a lot of information that we need about what's going on in different parts of the brain, which involves a straightforward analytical approach,"

Optical spectroscopy research team *(from left to right):* Dr. Pierre Paul Proulx, Dr. Guy Cormier, Dr. John A. Capobianco, Tania Peres, Pierre Kabro, Chedly Rejeb and Rafael Carazo.

states Pocock.

Biochemistry graduate student Katherine Bonter is currently working with Wise and Pocock. She is measuring the levels of glutamic acid — an excitatory neuro-transmitter — in the brains of live rats. Glutamic acid has an effect on the levels of dopamine in the brain, but the way in which that connection works is still unclear.

Organic Chemistry

Assistant Professor **Youla S. Tsantrizos**, who has a background in synthetic organic chemistry, natural products chemistry as well as biochemistry, is searching for new effective ways of fighting cancer and viral diseases. The focus of her research can be divided into three areas: natural products chemistry, biosynthesis and nucleic acid chemistry. However, the main goal of all three areas is the development of new chemotherapeutic agents which control or inhibit gene expression. Professor Tsantrizos explains that "...the uncontrolled proliferation of cancer cells is primarily due to the incorrect expression of genes (or the expression of incorrect genes) known as oncogenes. These types of genes are usually formed when protooncogenes (normally found in healthy cells) are damaged by a mutagen. Similarly, viral diseases involve the expression of the viral genes by the infected host. Therefore, it is not surprising that the mode of action of most antitumor and antiviral agents involves inhibition of DNA or RNA expression and that natural products which intercalate, cleave or covalently bind to DNA exert strong toxic effects on tumor cells or cells infected with a viral gene."

She continues saying that "...the healing and killing power of natural products have been exploited by humans since prehistoric times. To this day, nature is still the main source of important therapeutic agents (as well as molecular blue-prints for synthetic analogues), and some of the most important antitumor drugs are derived from microorganisms." Thus, one of the main areas of research activities in Professor Tsantrizos' laboratory is the area of natural products isolation, structure elucidation and biosynthesis. The expertise of her group in the area of purification and structure determination of novel bioactive compounds has been supported by 270 and 500 MHz Nuclear Magnetic Resonance spectros-

copy instruments which are available to her students at McGill University.

Due to the interdisciplinary nature of her work, Professor Tsantrizos maintains active collaborations with Professor **Paul Widden**, at the Biology Department of Concordia University, "an expert mycologist who provides us with rare microorganisms which he has identified," explains Tsantrizos. Her group has now obtained a number of unusual fungi from Dr. Widden's collection, and have begun screening them for their ability to produce antitumor and/or antiviral metabolites. In collaboration with **Dr. Brian Leyland-Jones** (Chairman, Oncology Department, Faculty of Medicine, McGill University) they have screened some of the most active cultures for their *in vitro* cytotoxicity against lung, colon, ovarian, breast and central nervous system, human carcinoma cell lines. "Some of our crude extracts exhibited activity in the same range, or *higher*, than some of the most important chemotherapeutic agents presently used for the treatment of cancer," says Tsantrizos.

The second important aspect of Tsantrizos' research is trying to develop synthetic gene fragments which specifically bind to defective genes or viral genes. She explains that "unfortunately, the majority of drugs used today for the treatment of cancer and viral disease do not selectively discriminate between oncogenes or foreign viral genes, and their normal counterparts, making the cure worse than the disease. In contrast, short synthetic polymers of 15-30 nucleotide units (known as antisense of antigene oligonucleotides) can be designed to recognize specific nucleic acid sequences which are unique to oncogenes, or viral genes, and bind to them with a high degree of selectivity," she says.

It is well known that nature provides us with a large variety of metabolites having strong affinity for DNA. These molecules damage DNA in a non-selective fashion exerting strong toxic effects on normal human cells, as well as tumor cells or cells infected with a viral gene. The ultimate goal of Professor Tsantrizos' group, is to combine the strong DNA-damaging properties of natural products with the selective interaction of synthetic oligonucleotides with DNA or RNA, in order to construct a new class of highly host-selective antitumor and antiviral therapeutic agents.

Accelerated DNA Testing

While Tsantrizos looks for cures, Assistant Professor **Susan Mikkelsen** has developed a device she hopes will speed up the process of diagnosing inherited diseases. It involves a relatively simple, three-step process that can produce results within 15 minutes. Current DNA tests can take up to a day.

Mikkelsen has managed to chemically attach a single strand of DNA to the surface of a carbon paste sensor on the end of an electrical conductor. The single strand is one that will bond with a DNA sequence associated with cystic fibrosis.

"The idea is that this sensor is then exposed to the DNA sample. If the complementary base sequence to what we have on the surface is present, then it will form an immobilized double strand on the surface," she explained.

If a double strand of DNA forms, that would indicate the presence of the disease being tested for. To determine whether or not bonding has taken place, the sensor is placed in a chemical compound that will bind to double-stranded, but not single-stranded DNA.

The next step, Mikkelsen said, is to "use an electrochemical technique that's sensitive to the surface of this compound. If the double strand is there, we get a high concentration near the surface. Our technique is very sensitive to what's near the surface of the electrode, so we wind up getting a high signal."

That signal implies a high concentration on the surface, and, therefore, the presence of double-stranded DNA.

Mikkelsen, an analytical chemist, is testing the device on a DNA sequence associated with cystic fibrosis. But it could theoretically be applied to a large number of diseases. "It would be applicable to anything that has DNA or RNA. That includes inherited disorders, and detection of a particular kind of bacteria or a particular virus," she said.

'...the healing and killing power of natural products have been exploited by humans since prehistoric times. To this day, nature is still the main source of important therapeutic agents (as well as molecular blue-prints for synthetic analogues), and some of the most important antitumor drugs are derived from microorganisms.'

'Members of the Department were just awarded a major grant from the federal government to purchase a mass spectrometer which will allow them to measure the molecular weights of proteins more accurately than ever before.'

Preliminary testing on the device has been done using synthetic DNA. Now Mikkelsen plans on using blood samples collected by the Montreal Children's Hospital during epidemiological screening for cystic fibrosis. She will then be able to compare the results achieved using the new sensor with those arrived at through standard testing techniques.

Doctoral student Kelly Millan has played a central role in the development of the device since she first started working with Mikkelsen in December 1990. "She's been great. It's so easy to get off the ground when you've got a really good first graduate student," Mikkelsen says.

"She did an undergraduate degree in biology, so we work more as a team since our backgrounds are so different. She contributed a lot to the project — a lot of techniques that she knew and that would have taken me a long time to learn."

Mikkelsen and Millan recently patented the sensor, which has been conceived, tested, and developed entirely at Concordia. They hope to be able to transfer the technology to a Quebec-based commercial diagnostic kit manufacturer.

Bioinorganic Chemistry

Professor **Ann English** works primarily in bioinorganic chemistry but is also involved in enzyme-based biosensor development with Mikkelsen. This work focuses on chemically modifying enzymes to make them electroactive and in characterizing the modified enzymes. The electroactivity of these enzymes is the key element in their sensing of physio- logically important compounds such as glucose and lactate. Two graduate students and two postdoctoral fellows are working with English and Mikkelsen on the project.

The main area of research for English, who has taught at Concordia since 1982, is studying heme proteins such as peroxidases. Some heme proteins, like hemoglobin and myoglobin carry and store oxygen. Heme peroxidases, on the other hand, remove hydrogen peroxide from organic systems by turning it into water. English wants to learn how the process works. "When these enzymes are acting as catalysts you have highly reactive intermediates involved. We don't really understand what controls their reactivity, and that is what we are trying to explore," she says.

Her group is currently working primarily with two peroxidases and comparing their chemistry to that of myoglobin. She explains that "we are trying to see how nature has designed myoglobin to reversibly bind oxygen, and the peroxidases to carry out their enzymatic function". The difference between the proteins lies in their polypeptides since the heme is identical in each case. Understanding this is important if we wish to do protein engineering.

To examine the peroxidases, they are first grown in English's lab in a genetically altered form of the bacterium *E. coli*. Then the proteins are isolated and purified. She noted that these preliminary steps are, in themselves, "a significant amount of work. After we have isolated the proteins, we do a number of chemical and physical studies on them. We use kinetics and a variety of spectral techniques," she says. "And, of course, we study their reactivity with peroxide and other substrates in detail."

English and other members of the Department were just awarded a major grant from the federal government to purchase a mass spectrometer which will allow them to measure the molecular weights of proteins more accurately than ever before. The mass spectrometer they will buy is accurate to 0.01 per cent. The level of accuracy is important, English says, because it means "you can now tell if you have altered your protein in any way. You couldn't tell that before, because previous techniques would only tell you the mass to plus or minus five or ten per cent". "We are very excited about getting this instrument and setting it up here," she adds. "There are not many people in academia who are using this technique yet."

English is also working on a research project funded by the Heart and Stroke Foundation. She is examining a newly-identified heme protein called guanylate cyclase involved in blood pressure control. When nitric-oxide — a com-

pound produced in the body — binds to the heme of this protein it causes a decrease in blood pressure. Because guanylate cyclase is only found in tiny amounts in the body, English is working on cloning it in *E.coli*. "This has not been done before. We want to get enough so that we can do physical and chemical studies on it possibly to understand how it works and control its activity, which may suggest new ways of regulating blood pressure. Hopefully we will succeed in getting the bacteria to make the protein," she states.

Capillary electrophoresis

Assistant Professor **Peter Banks**, a recent appointee, rounds out the Department's team of analytical chemists. His specialty is separation science involving peptides, proteins and amino acids.

Banks works primarily with a technique called capillary electrophoresis. He places a tiny silica capillary in a quartz sheath flow cuvette, then pumps a microsample of protein solution down the bore of the cuvette and along the capillary. After the proteins have passed through the capillary, Banks shines a laser through it to collect fluorescence — the radiation emitted by what he called "excited biomolecules."

He is using the technique in immuno-assays, in which antibodies form a complex with a specific type of analyte. He will tag the antibody with a fluorescent 'label.' Some of the antibody will then complex with the analyte, and the rest will remain free.

"So I use capillary electrophoresis to separate out the complex, which contains the analyte and the antibody, from the free antibody, and that way I'm able to directly quantitate the analyte," he explained.

The technique is extremely fast — it can produce results in under ten minutes — and is very sensitive. Banks said he hopes to be able to detect zeptomoles, or about 600 molecules at a time. There are some problems with capillary electrophoresis — the proteins tend to absorb on the silica capillaries for instance — but Banks is confident he can overcome them and achieve a high level of sensitivity.

"My bottom line is that I want a fast assay and I want a very sensitive assay. That's what I'm striving for," he said.

'What we want is the gene coding for our protein of interest because we want to mutate the gene, so we change the protein in a known way'

Protein Targeting

Biochemist **Paul Joyce** is doing fundamental research in "trying to understand one of the basic problems of cell biology." He hopes to determine what targets a protein to travel to a specific site in a cell. For example, most of the energy production in cells takes place in the mitochondrion. The mitochondrion requires the presence of proteins to produce that energy. However, most protein synthesis takes place elsewhere in the cell. Nobody knows exactly how these proteins travel from the site of their production within the cell to the locales at which they perform their specific functions. "I am working on plant cells, and the reason they are interesting is that they have chloroplasts in addition to mitochondria and nuclei", he says. "The important thing is that it makes another locale to which you can target proteins.

Joyce speculates that "there must be information in the protein itself, some sort of targeting sequence that somehow interacts with something else in the cell to get it to the proper location". But in addition to these signals, there must be receptors at the destination site as well. This would mean that, for instance, the nucleus only recognizes proteins with nuclear signals, mitochondria only recognizes proteins with mitochondrial signals, etc., "I am trying to more precisely define the signals and what the receptors actually are, and why the receptors work only for specific signals", says Joyce. For example, many similar proteins with similar targeting signals are found in the chloroplast and mitochondrion. This means that the receptors for them must be extremely sensitive to ensure that these proteins reach their proper intracellular destinations and are not mislocalized. A crucial part of Joyce's research is understanding the structure of the proteins he is interested in. "What we want is the gene coding for our protein of interest because we want to mutate the gene, so we change the protein in a known way," he says. By mutating the gene, Joyce and the graduate students he works with can examine how specific changes affect whether or not the protein reaches its destination in the cell.

The diversified and interdisciplinary research projects outlined and planned for the future act as catalysts for the continued growth of the international reputation of faculty and graduate students in the Department of Chemistry and Biochemistry.

For further information, please contact:

Graduate Programme Director
Dept. of Chemistry and Biochemistry
1455 de Maisonneuve Blvd. W.
Montreal, Quebec H3H 1M8
Telephone: (514) 848-3373
Fax: (514) 848-2868

EDITORIAL COMMITTEE:
Chair: D. Mankiewicz
Editor: D. MacFadden
Writer: P. Moscovitch

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LOCATION:
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Tel. (514) 848-3808,
Fax: (514) 848-2812